

X-RAY EFFECTS ON ADULT ARTEMIA

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The available information concerning the lethal dosage of ionizing radiations in animals is limited to a certain few microorganisms, selected insects and laboratory types of vertebrates. Even more limited has been research on radiation-induced sterility in females. Because there are extreme differences between vertebrate results and those with holometabolous insects, it is of interest to fill in the gaps by extending research to a wide variety of representative animals. The present paper introduces a member of a primitive order of crustacea to this form of research. Except for attempts to induce visible mutations (Gajewskaja, 1923), we have been unable to find previous reports of a radiobiological nature on *Artemia*.

MATERIALS AND METHODS

Commercial, dry eggs of the California strain (diploid amphigonic) of *Artemia salina* Leach, obtained from the M.B.L. Supply Department, were hatched in sea water and reared to maturity in large battery jars. Evaporation in our basement laboratory at Woods Hole was extremely slight; replacement was with sea water as is the standard procedure in bulk culturing. Cultures were fed daily with a few drops of either homogenized liver paste (commercial baby food) or yeast suspension unless the growth of natural microorganisms was obviously abundant. As soon as sex characteristics were distinguishable, individuals were segregated into male and female holding jars. When the accumulated total was adequate to provide five or more groups of at least five adult animals, experiments were set up. In this way animals were used within three days after reaching maturity.

The x-ray generator was that of the M.B.L., Woods Hole. This has two water-cooled G. E. (XTP) tubes in opposed position and operates at 30 ma and a 200 kv peak with an inherent filter equivalent to 0.2 mm. of copper. Air circulation in the room is assured by a fan.

Artemia were exposed 5 at a time in small plastic containers (4000 cu mm.). Exposures below 5000 r were made at an intensity of 2550 r per minute. Higher doses were delivered at 6120 r per minute. Calibration was achieved by M. Berman by inserting a rubber-covered ionization chamber through a hole bored in the side of one of the plastic containers which was then filled with sea water. Control samples of *Artemia* were held in exposure chambers in the anteroom for a period equalling that of the longest experimental treatment.

After irradiation, *Artemia* were maintained in a 30° C. incubator where evapo-

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ration was not a problem because the air was kept near the saturation point. Those used for life span records were segregated by sex and maintained 5 per wide mouth jar (50 by 80 mm.). For sterility studies, individual females were placed in stender dishes (60 by 35 mm.) and each provided with an untreated male. Regular mating associated with internal fertilization was observed periodically with all pairs. Feces, debris, and dead animals were removed daily by pipette and an equal amount of fresh sea water was added. If ova or nauplii had been deposited the pair of parents were transferred by glass tube to another stender dish. All incubator *Artemia* were fed daily by adding dilute yeast suspension in the amount of one drop per animal to the water of the container.

RESULTS AND CONCLUSIONS

Figure 1 presents survival of *Artemia* after exposures to various doses of x-rays. As shown, a sex difference was revealed at higher exposures by the tendency for females to die earlier than males in simultaneous experiments.

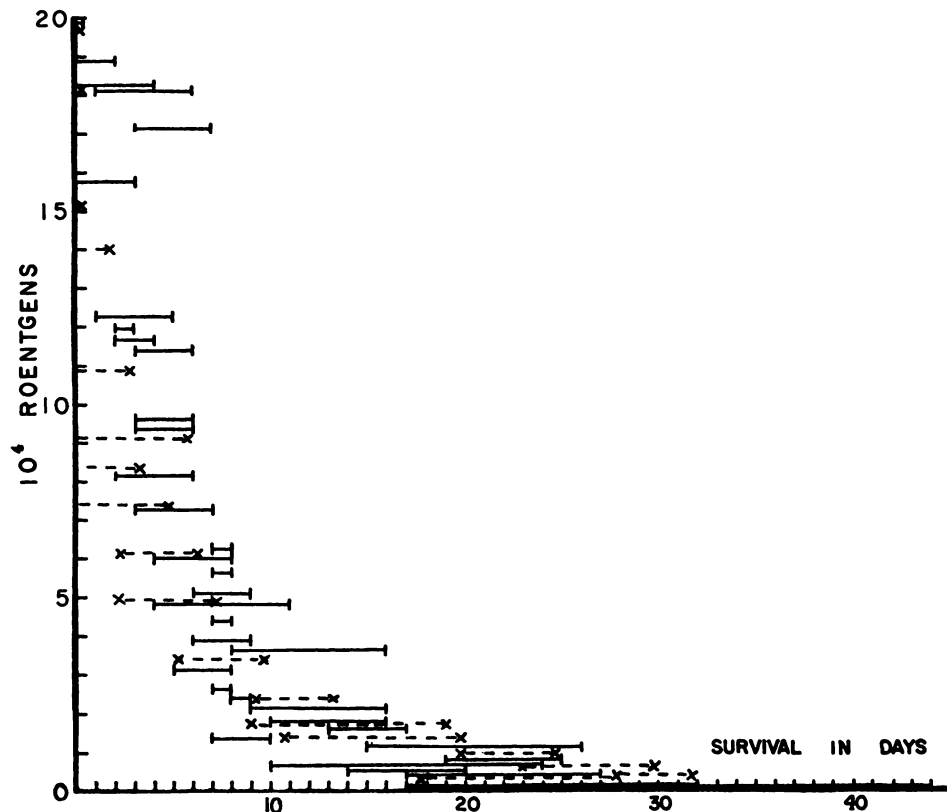


FIGURE 1. The range in time during which deaths in samples of five *Artemia* occur subsequent to x-ray exposure. Male records are designated by unbroken lines and short vertical marks; female records by broken lines ending in x marks. Control records are indicated by the thickening of the base line.

Furthermore, at 150,000 r and above, females failed to recover, although many males were able to survive for several days provided the exposure did not exceed 200,000 r. As a precaution, in the event that *Artemia* would be able to recover from "knock down" doses as do adult insects, the following were delivered above those plotted: 224,910 r, 257,040 r, 289,170 r, 321,300 r, 353,430 r, and 358,785 r. No recovery occurred.

The minimal dosage at which all animals are killed seems to be the most feasible approach to lethal dose with materials of this type. As shown in Figure 1 for doses above 10,000 r, the variability in the time of death is not great when observations are taken in days for an organism whose life span is days. This is considerably different than the picture obtained with mammals whose life span is years, even decades, and the standard approach of 50% dead in 30 days provides a stable centering point for a high degree of variability. A 50% curve could be drawn for each sex through the ranges shown of Figure 1 and its shape would be

TABLE I
Summary of experiments on gamete deposit per individual female Artemia

X-ray dose in r	Highest no. of broods	Average no. of broods	Highest total no. of gametes	% Viviparous	Average no. of gametes	No. of females	Average adult life span in days
0	6	4.6	287	46.40	67.17	20	16.9
510	4	2.0	140	48.25	71.50	15	12.4
1,020	5	3.0	176	42.77	37.82	20	10.5
2,040	4	2.0	138	.04	57.46	15	9.2
3,060	2	0.9	130	.01	50.78	10	15.4
4,080	2	1.0	67	.14	29.40	10	10.7
5,160	2	0.7	55	0	18.29	15	5.9
11,527	1	0.7	79	0	31.40	5	3.4
15,900	1	0.5	23	0	6.60	5	9.8
21,200	1	0.4	51	0	12.60	5	6.6

the typical steep S. However no length of survival time suggests itself as particularly significant.

Table I presents a summary of observations on gamete production by females subsequent to their exposure. It is based upon the deposit of 46,966 ova and nauplii by 120 females. An initial experiment clearly demonstrated that no reproductive units except those pre-formed would be discharged after exposures above 5160 r and the groups of 5 experimental animals at each dose were not supplemented by replication. At 4080 r and 5160 r, exceptional individuals deposit a second meager brood but the great majority of females so treated die with empty uteri. At 3600 r second broods are adequate and death with a full uterus is not uncommon. Observations at the next lower dose, 2040 r, made it apparent that the sterility threshold had been bracketed. A comparison of the average life spans of Table I with the ranges at similar doses in Figure 1 demonstrates that deaths occurred relatively early in the sterility investigation. A number of cases of death of either or both parents following transfer lead us to suspect that brine shrimp are too delicate for repeated handling. Thus a number of females potentially able to give rise to additional broods of offspring may have been killed by

mechanical injury. Top individual performance as included in the table may be more valid as a basis for expectancy under normal conditions than are the averages obtained here. On either basis, the neighborhood of 2000 r is critical not only for numbers of broods produced but on the basis of inhibition of viviparity.

Normally, as occurred in controls, *Artemia* females void either live nauplii or brown heavy-shelled eggs (Lochhead and Lochhead, 1940). In addition to these, the experimentals produced several unusual types of gametes: partially formed nauplii, smooth shiny white eggs, dull white ragged eggs, orange eggs and tan eggs. Partially formed nauplii were able to complete development but none of the other types were seen to hatch. This may be due in part to their vulnerability through lack of a protective shell. Protozoa and copepods were seen actively attacking, presumably feeding on shell-less eggs.

Hatchability of brown shelled eggs will not be considered here. The data on various batches are not all identical because ways of handling this type of product were being explored.

DISCUSSION

The lethal doses of ionizing radiation vary considerably among different types of animals. Best investigated from this standpoint have been the mammals where the total body dosage required to kill 50% of the adults within 30 days ranges from 200 to 800 r (Patt and Brues, 1954). To kill such types within hours after exposure requires higher doses, even as much as 25,000 r to 50,000 r for extreme cases (Hagen and Sacher, 1954; Patt and Brues, 1954). Massive as such doses may seem, they fall far below those producing immediate lethality for certain bacteria and protista on the one hand (Bonham *et al.*, 1947) and for adult insects on the other (Hassett and Jenkins, 1952; Sullivan and Grosch, 1953). The present results, 150,000 r for females and 200,000 r for males, place *Artemia* near but below holometabolous insects in radiation tolerance as adults. Demonstration of a sex difference was not unexpected. However females are usually found more resistant than males in the various animals studied.

Physiological factors undoubtedly are of importance in determining radiosensitivity and radiotolerance. One mammal, the bat, shows no effect on life span under 15,000 r just after hibernating and survives for several days after 60,000 r (Smith *et al.*, 1951). In work with insects the senior author has been impressed with the resistance of cell types specialized for physiological activity. Often this specialization is reflected cytologically in some degree of somatic polyploidy, a situation achieved through endomitosis and conceived to confer radioresistance (Grosch and Sullivan, 1954). Consistent with this view are the present findings in light of the fact that endomitosis accompanies differentiation to adult condition of a number of important *Artemia* tissues (Barigozzi, 1942). At earlier stages when mitosis is occurring and before endomitotic tissues have attained multiples of the basic chromosome number, *Artemia* should prove radiosensitive. Indeed, one of Gajewskaja's (1923) statements suggests this. Furthermore, even the most tolerant forms of holometabolous insects have radiosensitive developmental stages (for *Habrobracon* see Clark and Mitchell, 1952), while mammals on the other hand never achieve a radiotolerant stage. The latter depend upon mitotically active tissue to prevent loss of physiological fluids and for maintenance of the lines of bodily defence.

The most useful study found for comparison with *Artemia* results has been that of Henshaw (1939) on the cut branches of the colonial coelenterate, *Obelia*. Here 250,000 r were required to suppress hydranth activity and growth of the differentiated unit whereas only 6% of that large dose suppresses initiation and halts growth in the bud stage, a period characterized by cell proliferation. Henshaw used the same MBL generator employed for the present work.

Unlike the lethal dose response, the level of x-ray treatment causing *Artemia* sterilization is not appreciably above that required in some vertebrates for complete, irreversible destruction of ovaries. For example, although the 30-day lethal total-body dose in rats produces great degenerative changes in the ovaries, as much as 3000 r may be required in local exposure to insure permanent sterility (Lacassagne and Gricouroff, 1941). This may also be true for rabbits and guinea pigs. A similar dose, which as shown above affects *Artemia* gamete production profoundly, produces only brief temporary sterility in the wasp, *Habrobracon* (Grosch and Sullivan, 1954). To completely sterilize this wasp 5000 r is required. Glücksmann (1947) cites the same figure for *Drosophila*. Both of these holometabolous insects have a polytrophic type of ovariole in which large groups of polyploid nurse cells accompany each oocyte. Therefore on histological grounds the ovaries are not directly comparable to other forms. Evidence that insect ovaries of a simpler structure may be vulnerable at much lower doses is supplied with results with grasshopper nymphs (Tahmisian and Vogel, 1953) which have a panoistic type of ovariole. For this relatively simpler and more generally comparable tissue, 350 r is adequate to destroy all but the most advanced eggs and 800 r results in complete destruction of the ovaries. This is much like the traditional range for mammalian dosage, man and mouse, summarized by Glücksmann (1947). At present we can only speculate concerning the feature responsible for the dose requirement in *Artemia* sterilization. It seems likely that the inter-related shell gland may be involved. Further investigation along these lines is indicated since there is a notable lack of agreement in the literature on why reproduction is sometimes viviparous and at other times oviparous. In order to avoid mechanical damage to adults the more tedious transfer of gametes is suggested in future experiments.

SUMMARY

As adults, *Artemia* males are killed by 200,000 r of x-radiation; females by 150,000 r. This approaches the doses required to kill adult holometabolous insects. Neither insects nor the brine shrimp depend upon mitotic tissues in adulthood. The sterility dose at 2000–3000 r for females is lower than in insects with polytrophic ovarioles and higher than in grasshoppers with simpler panoistic ovarioles. Further investigation is suggested for the interrelations of the *Artemia* shell gland and the reproductive cycle.

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